16. (Amended) The method of claim 11, wherein the cell is a plant cell.

20. (Amended) The method of claim 12, wherein, in the method for producing the SATAC, the cell is a plant cell.

REMARKS

A check for the fee for a three month extension of time accompanies this response. Any fees that may be due in connection with filing this paper or with this application during its entire pendency may be charged to Deposit Account No. 50-1213. If a Petition for extension of time is required, this paper is to be considered such Petition, and any fee charged to Deposit Account No. 50-1213.

Claims 1, 4, 6, 7, 9-27 and 29-32 are pending in this application. Claims 2, 3, 5, 8, 28 and 33, which are withdrawn from consideration as being drawn to non-elected subject matter, are cancelled herein. Applicant reserves the right to file divisional applications to the non-elected subject matter.

Claims 9, 10, 13-15, 17-19, 21-28, 31 and 32, which are also withdrawn from consideration as being drawn to non-elected subject matter, are retained herein. As discussed below and as set forth in the Restriction Requirement mailed July 16, 2002, in connection with the above-captioned application, the aforementioned claims are linking claims, which must be examined with the claims of the elected group. If any linking claim is deemed allowable, then the Restriction Requirement must be withdrawn as to the linking claims and any claim including limitations thereof.

Claim 11 is amended to correct a minor typographical error and to more particularly point out the claimed subject matter. The amendments find basis in the specification, *e.g.*, at page 29, line 11, through page 30, line 2, and at page 33, line 19, through page 34, line 27. Claims 16 and 20 are amended to remove reference to non-elected subject matter. No amendments have been made to obviate prior art and no new matter has been introduced.

A marked up copy per 37 C.F.R. §1.121 of the amended claims is attached to this response.

WITHDRAWAL OF CLAIMS 9, 10, 13-15, 17-19, 21-28 and 31-33 FROM CONSIDERATION AS BEING DRAWN TO NON-ELECTED SUBJECT MATTER

Claims 9, 10, 13-15, 17-19, 21-28, 31 and 32 are withdrawn from consideration as being directed to non-elected subject matter. These claims, lik3 Claims 1, 11 and 12, that have been examined in setting forth the instant Office Action, are linking claims. The Restriction Requirement mailed July 16, 2002, in connection with the above-captioned application, acknowledges that Claims 1, 9-15, 17-19, 21-27, 31 and 32 link the subject matter of Groups I-IV. The Restriction Requirement was subject to non-allowance of the linking claims.

According to MPEP §809, when claims linking more than one group are found, the linking claims **must** be examined with the elected group; if the linking claims are deemed allowable, then the Restriction Requirement must be withdrawn and all claims directed to nonelected subject matter which depend from or include all the limitations of the linking claims must be rejoined.

Therefore, it is respectfully submitted that Claims 1, 9-15, 17-19, 21-27, 31 and 32 must be examined along with the claims of Group I (Claims 4, 6, 7, 16, 20, 29 and 30) that were elected responsive to the Restriction Requirement of July 16, 2002. Of the aforementioned linking claims, only claims 1, 11 and 12 have been examined in setting forth the instant Office Action. Therefore, the remaining linking claims, 9, 10, 13-15, 17-19, 21-28, 31 and 32, are retained herein pending reconsideration of their withdrawal.

OBJECTION TO CLAIMS 16 AND 20

Claims 16 and 20 are objected to as being drawn to non-elected subject matter. This objection has been rendered moot by amending the claims to remove reference to the non-elected subject matter.

THE REJECTION OF CLAIMS 11, 12, 16 and 20 UNDER 35 U.S.C. § 112, Second Paragraph

Claims 11, 12, 16 and 20 are rejected under 35 U.S.C. § 112, second paragraph as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter. Specifically, the Office Action alleges that it is unclear how the final method step of "selecting a cell" relates to the steps of "introducing a DNA fragment" and "growing the cell" in the overall flow of the method steps. Reconsideration of this rejection is respectfully requested in view of the amendments herein and the following remarks.

RELEVANT LAW

Claims are not read in a vacuum but instead are considered in light of the specification and the general understanding of the skilled artisan. *Rosemount Inc. v. Beckman Instruments, Inc.*, 727 F.2d 1540, 1547, 221 USPQ 1, 7 (Fed. Cir. 1984), *Caterpillar Tractor Co. v. Berco, S.P.A.*, 714 F.2d 1110, 1116, 219 USPQ 185, 188 (Fed. Cir. 1983). When one skilled in the art would understand all of the language in the claims when read in light of the specification, a claim is not indefinite.

There are no requirements for terms to be defined in the claims when one of skill in the art can readily determine the meaning of the term based on the description and definitions provided in the specification. In this respect, an applicant is entitled to be its own lexicographer [see, *e.g.*, MPEP 2111.01 "Applicant may be his or her own lexicographer as long as the meaning assigned to the term is not repugnant to the term's well known usage and utilize terms within the claims that are clear from a reading of the specification. *In re Hill*, 73 USPQ 482 (CCPA 1947)."]. When applicant has provided definitions in the specification, the claims are interpreted in light of such definition.

35 U.S.C. § 112, second paragraph requires only reasonable precision in delineating the bounds of the claimed invention. Claim language is satisfactory if it reasonably apprises those of skill in the art of the bounds of the claimed

invention and is as precise as the subject matter permits. *Shatterproof Glass Corp. v. Libby-Owens Ford Col.*, 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir.), cert. dismissed, 106 S.Ct. 340 (1985).

The amount of detail required to be included in the claims depends on the particular subject matter and the prior art and is not to be viewed in the abstract, but in conjunction with whether the specification is in compliance with the first paragraph of 35 U.S.C. § 112. If the claims, read in light of the specification, reasonably apprise those skilled in the art of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more:

> [i]t is not necessary that a claim recite each and every element needed for the practical utilization of the claimed subject matter (*Bendix Corp. v United States*, 600 F.2d 1364, 1369, 220 Ct. Cl. 507, 514, 204 USPQ 617, 621 (1979); See, also, *Carl Zeiss Stiftung v. Renishaw plc*, 20 USPQ2d 1094, 1101).

ANALYSIS

Claim 11 is amended to more particularly point out the claimed subject matter. As amended, the step of "growing the cell under selective conditions...." specifies that selective growth is performed "whereby a satellite artificial chromosome is produced." This amendment finds basis in the specification, *e.g.*, at page 29, line 11, through page 30, line 8, and at page 33, line 19, through page 34, line 27. The cited passage(s) describe in great detail methods for producing satellite artificial chromosomes (SATACs) by growing cells under selective conditions, and the types of selective conditions. For example, the cited passage(s) describe the production of satellite artificial chromosomes where heterologous DNA is introduced into a cell and integrated into the pericentric heterochromatin of a native chromosome, resulting in a multicentric chromosome. As described in the specification, growth of cells

under selective conditions results in the amplification event(s) that lead to production of satellite artificial chromsomes.

Furthermore, attention is directed to U.S. Patent No. 6,077,697, which is based upon a parent application of the instant application, in which claim 1 recites:

> 1. A method, comprising:
> introducing one or more DNA fragments into a cell, wherein the DNA fragment or fragments comprise a selectable marker;
> growing the cell under selective conditions to produce cells that have incorporated the DNA fragment or fragments into their genomic DNA; and
> selecting a cell that comprises a satellite artificial chromosome.

Accordingly, the language of claim 11 as filed, and as amended is presumptively clear and definitite.

Thus, claim 11 is clear such that one of skill in the art would understand the metes and bounds of the claim as read in light of the specification. Therefore, as amended, claim 11 and claims dependent thereon (claims 12, 16 and 20) are not indefinite.

THE REJECTION OF CLAIMS 1, 4, 6, 7, 11, 12, 16, 20, 29 and 30 UNDER 35 U.S.C. §112, FIRST PARAGRAPH

Claims 1, 4, 6, 7, 11, 12, 16, 20, 29 and 30 are rejected under 35 U.S.C. § 112, first paragraph because it is alleged that the specification, while being enabling for a mammalian satellite artificial chromosome in a mammalian cell, does not reasonably provide enablement for the preparation of a satellite artificial chromosomes of <u>any</u> species in <u>any</u> cell. In particular, the Office Action alleges (1) that the differences between plants and animals, particularly their respective satellite DNA, centromeres and heterochromatin, make it unpredictable that a plant would have centromeres that are structurally and biochemically the same as those of animals; and (2) that the alleged complexity

of satellite artificial chromosomes, especially their large sizes, when combined with other components of heterologous expression constructs such as different promoters, enhancers, codon optimization, termination regions and other regulatory regions, would lead one of skill in the art to expect that a satellite artificial chromosome constructed for mammalian cells would differ from a satellite artificial chromosome that is constructed for a plant cell. To support this allegation, the Office Action cites numerous (post filing date) references that allegedly demonstrate the differences between plant and animal DNA and chromosomal components and the difficulties in transferring large pieces of DNA between cells and (see, *e.g.*, Ferl *et al.* in Buchanan *et al.* "*Biochemistry and Molecular Biology of Plants*" (2000) American Society of Plant Physiologists, Rockville MD, pg. 324; Lehninger "*Biochemistry*", 2nd Edition (1976) Worth Publishers, New York, pg. 864; Willard *Science 290:* 1308-1309 (2000); Copenhaver *et al. Science 286:* 2468-2474 (1999); Shen *et al. Current Biology 10:* 31-34 (2000); Telenius *et al. Chromosome Res. 7*: 3-7 (1999); Avramova *et al. Plant Physiology 129:* 40-49 (2002); Brown *Trends in Biotech. 18:* 403 (2000); Perez *et al. Trends in Biotech 18*: 402-403 (2000); and Hadlaczky *Curr. Opin. Mol. Ther. 3:* 125-132 (2001)). The Office Action further alleges that while the specification provides methods for the preparation and transfer of an animal satellite artificial chromosome into a mammalian cell (human, mouse and hamster cells), there is no evidence that these methods do not produce a satellite artificial chromosome from any source (*i.e.*, plants) that is operable in any cell type (*i.e.*, a plant cell). It is alleged that while one of skill in the art can readily make changes to Applicant's animal satellite artificial chromosomes to generate a non-animal satellite artificial chromosome, there is no guidance as to what these changes should be, leading one of skill in the art to make random changes fraught with trial and error, requiring undue experimentation. To support this allegation, the Office Action cites Willard (*Science 290:* 1308-1309

(2000)) that allegedly demonstrates the state of the art of artificial chromosome technology.

The Office Action concludes that the specification is not enabling for a satellite artificial chromosome that is universally adapted to be operable in all cell types. The Office Action further concludes that, due to the unpredictability that the satellite artificial chromosome provided by Applicant would be operable in all cell types, Applicant has only enabled a satellite artificial chromosome for use in a mammalian cell and not in any cell type. This rejection is respectfully traversed.

First it is noted that the instant application is a continuation of copending U.S. application Serial No. 08/835,682, filed April 10, 1997, is also a continuation-in-part of U.S. application Serial No. 08/695,191, filed August 7, 1996, now U.S. Patent No. 6,025,155, is also continuation-in-part of U.S. application Serial No. 08/682,080, filed July 15, 1996, now U.S. Patent No. 6,077,697, and is also a continuation-in-part of copending U.S. application Serial No. 08/629,822, filed April 10, 1996. The presently pending claims have an effective filing date of April 10, 1996.

Reliance upon post-filing date references to establish a lack of enablement is improper. Furthermore, reliance upon references such as Willard *et al.* and references that describe other artificial chromosomes, is inapt, since the technology therein is unrelated to the technology upon which the instant methods are based, and does not represent the state of the art of SATAC technology. It is the instant applicant who first invented the satellite artificial chromosomes and they can be produced by a method, whose steps are outlined in detail in the specification, in any eukaryotic cell that contains chromosomes with amplifiable DNA; one does not have to know what components are required or what telomeres are needed or other parameters. Cells, when treated as described in the instant application, generate chromosomal structures through an ampilfication event that result in chormosomal structures that

produce SATACs. Knowledge of the mechanism is not needed. Furthermore the amplification event that leads to generation of SATACs does occur in plants (see, *e.g.*, U.S. Patent Nos. 6,355,860 and 6,100,092, which demonstrate such event).

The instant applicant is the first to identify such events and their use to generate SATACs. As such, this is a pioneering invention entitled to broad scope.

Relevant law

To satisfy the enablement requirement of 35 U.S.C § 112, first paragraph, the specification must teach one of skill in the art to make and use the invention without undue experimentation. Atlas Powder Co. v. E.I. DuPont de Nemours, 750 F.2d 1569, 224 USPQ 409 (1984). This requirement can be met by providing sufficient disclosure, either through illustrative examples or terminology, to teach one of skill in the art how to make and how to use the claimed subject matter without undue experimentation. This clause does not require "a specific example of everything *within the scope* of a broad claim." In re Anderson, 176 USPQ 331, at 333 (CCPA 1973), emphasis in original. Rather, the requirements of § 112, first paragraph "can be fulfilled by the use of illustrative examples **or** by broad terminology." In re Marzocci et al., 469 USPQ 367 (CCPA 1971)(emphasis added).

Further, because "it is manifestly impracticable for an applicant who discloses a generic invention to give an example of every species falling within it, or even to name every such species, it is sufficient if the disclosure teaches those skilled in the art what the invention is and how to practice it." In re Grimme, Keil and Schmitz, 124 USPQ 449, 502 (CCPA 1960). Thus, there is no doubt that a patentee's invention may be broader than the particular embodiment shown in the specification. A patentee not only is entitled to narrow claims particularly directed to the preferred embodiment, but also to

broad claims that define the invention without a reference to specific instrumentalities. Smith v. Snow, 294 U.S. 1, 11, 24 USPQ 26, 30 (1935).

Thus, there is no requirement for disclosure of every species within a genus. Applicant is entitled to claims are commensurate in scope not only with what applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the applicant has disclosed.

The inquiry with respect to scope of enablement under 35 U.S.C. §112, first paragraph, is whether it would require undue experimentation to make and use the claimed invention. A considerable amount of experimentation is permissible, particularly if it is routine experimentation. The amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims. Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986); see also In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988).

ANALYSIS

As demonstrated below, it would not require undue experimentation to produce satellite artificial chromosomes in plants, and introduce them into the different cell types, including plant cells, that are within the scope of the claims, in view of the level of skill in the art and the teachings and disclosure in the specification regarding methods for generating satellite artificial chromosomes for use in different species; methods for cloning centromeres from difference species for use in the preparation of species-specific satellite artificial chromosomes; and methods for producing cells of different species that contain heterologous nucleic acids such as satellite artificial chromosomes.

As demonstrated below, the teachings of the specification, when taken in conjunction with what is known to one of skill in the art, are such that it would require not undue experimentation to perform the steps of the methods claimed herein, including the preparation and isolation of plant satellite artificial chromosomes and their introduction into a cell of any species to produce cells containing a satellite artificial chromosome.

The level of knowledge and skill in the construction, introduction into cells and stable expression of large sizes of DNA was so high as of the effective filing date that it would not have required extensive experimentation by one of skill in produce plant satellite artificial chromosomes by the methods in the working examples and the publications incorporated herein by reference, nor would it have required extensive experimentation to transfer the resulting plant artificial satellite artificial chromosome into a plant cell. Furthermore, the specification describes methods for generation and identification of SATACS, exemplifies such methods with respect to mammalian chromosomes, and provides a broad disclosure that can be practiced with any eukaryotic cell. It is only necessary to introduce a piece of DNA and a selectable marker into a cell, grow the cell under selective conditions, look for cells that contain SATAC, select such cells, and isolate a SATAC therefrom. There is no evidence of record that suggests that such events are unique to animal cells, nor is there any reason to believe such. On the contrary the specification teaches that the events and the methods based thereon are applicable to cells from any eukaryotic species. As noted above, the generation of a SATAC requires an amplifcation event and there is no reason provided by the Examiner nor of record that suggests that plant chromosomes do not undergo amplification. In fact, plants do have amplifiable regions (see, *e.g.*, U.S. Patent Nos. 6,355,860 and 6,100,092 attached hereto).

Scope of the claims

The claims are directed to methods for producing a cell that contains heterologous nucleic acid by introducing a satellite artificial chromosome into the cell (claims similar thereto have issued to the instant applicant). The dependent claims specify that the cells are plant cells and also that the satellite artificial chromosomes are those produced in plants (*e.g.*, plant satellite artificial chromosomes, such as tobacco, rice and maize satellite artificial chromosomes); and particular methods for the production of the satellite artificial chromosomes.

Teachings of the specification

The specification describes the production, characterization and isolation of satellite artificial chromosomes and their transfection into cells. The teachings of the specification describe how to: (i) prepare satellite artificial chromosomes with heterologous nucleic acid incorporated therein, (ii) transfer the satellite artificial chromosome containing the heterologous nucleic acid into a cell; and (iii) determine the expression of a gene product(s) encoded by the satellite artificial chromosome.

Each of these steps are described in detail in the specification. In addition, the specification provides numerous working examples of the procedures and results involved in the claimed methods. For example, he specification discloses methods for generating artificial chromosomes, such as satellite artificial chromosomes; methods for generating species-specific satellite artificial chromosomes; methods for cloning centromeres from particular sources; methods for isolation and large-scale production of artificial chromosomes; methods for delivery of artificial chromosomes to selected cells, including, for example, the cells of a host plant, animal or insect; and methods for the expression of products encoded by the nucleic acids of the artificial chromosomes in cells, including the cells of a host animal, plant, or insect. Further, the specification provides cell lines and chromosomes produced by the methods described, which can be used as vehicles for the expression of heterologous nucleic acids in cells *in vitro* and *in vivo*.

The specification provides methods of generating satellite artificial chromosomes, and characterizes in exquisite detail the artificial chromosomes generated by such methods. To illustrate the methods and products thereof, the specification describes the exact procedures used to generate multiple specific cell lines containing SATACs (see, *e.g.*, Examples 2-7, beginning at page 75, line 8), and Applicant provides to the public no less than six of the described cell lines that have been deposited at an authorized depository (*i.e.*, the European Collection of Animal Cell Culture) (see, *e.g.*, page 74, line 23, through page 75, line 7).

The specification teaches the development of a satellite artificial chromosome containing sequences that can be of animal or plant origin and contain centromere-related sequences. For example, the specification provides methods for generating species-specific satellite artificial chromosomes by adding a centromere from other species, including plants (see, *e.g.*, at page 12, lines 3-16). In the specification, *e.g.*, at page 11, line 30, through page 12, line 16, a method for cloning a centromere in a selected species (*e.g.*, plants) is described. These methods for cloning centromeres from a selected animal or plant include: (i) preparing a library of DNA fragments that contains the genome of the plant or animal; (ii) introducing each of the fragments into a mammalian satellite artificial chromosome that contains a selectable marker and a centromere from a species different from the selected plant or animal; (iii) introducing each of the satellite artificial chromosomes into a cell, which is grown under selective conditions; and (iv) selecting cells containing satellite artificial chromosomes. Satellite artificial chromosomes that are identified by the methods provides herein should contain a centromere encoded by the DNA from the library, be it plant DNA or mammalian DNA, and should contain the necessary elements for stable replication in the selected species. Thus, the specification provides methods where a satellite artificial chromosome is

developed in one source cell, modified to contain sequences specific to the centromere region of a target cell and then transferred to the target cell type.

Methods for the modification of satellite artificial chromosomes are described in extensive detail in the specification (*e.g.*, at page 39, line 26, through page 40, line 21; and page 150, through page 157). The cited passage(s) describe the use of homologous recombination to insert new DNA fragments into a satellite artificial chromosome. Accordingly, methods to generate and modify a satellite artificial chromosome to contain a plant centromere DNA sequences have been provided.

The specification further teaches methods for producing satellite artificial chromosomes that contain heterologous DNA and the expression of the heterologous DNA contained therein in cells (see, *e.g.*, page 39, line 25, through page 41, line 3; page 61, line 28, through page 62, line 7; page 150, line 1, through page 165, line 12 and Example 12 beginning on page 140). Procedures for the isolation of artificial chromosomes (see, *e.g.*, page 41, line 4, through page 42, line 3; page 32, lines 13-24; page 80, lines 20-27; and Example 10, beginning on page 124) and for the transfer of the artificial chromosomes into cells (see, *e.g.*, page 10, lines 25-31; page 48, line 11, through page 51, line 26; page 52, line 11, through page 55, line 3; page 70, line 14, through page 72, line 27; and Example 13 beginning on page 165) are also described in detail in the specification.

At page 54, line 1, through page 55, line 3, the specification describes how to introduce SATACs into plant cells by methods, such as direct transfer of DNA by processes, such as PEG-induced DNA uptake, protoplast fusion, microinjection, electroporation, and microprojectile bombardment, such as particle gun bombardment.

The satellite artificial chromosomes contained within the cells were extensively characterized using methods including Southern hybridization, long-range mapping of restriction endonuclease sites, indirect immunofluorescence

with anti-centromere antibodies, *in situ* hybridization, analysis of G-band patterns, and chromosome painting. Such extensive analysis provides definition of the satellite artificial chromosomes at the level of the basic structural and functional elements of these chromosomes, including repeated units of satellite and foreign DNA. Many of these features are depicted schematically in Figures 1-3 of the application.

By following the methods set forth in the specification, the specification teaches one of skill in the art how to generate SATACS from various species (*e.g.*, plants and animals); readily identify the resulting satellite artificial chromosomes based on the detailed characterization provided in the specification; incorporate foreign nucleic acid (*e.g.*, heterologous DNA encoding a product into an artificial chromosome) and isolate and transfer artificial chromosomes into cells from various species (*e.g.*, plants and animals). Thus, the teachings of the specification provide how to make and use the satellite artificial chromosomes and to combine these artificial chromosomes with known recombinant DNA procedures, many of which are referenced in the specification, to achieve any number of particular outcomes, including the introduction and long-term expression of nucleic acids encoding products in cells of host animals, plants and insects.

Level of Skill

The level of skill in this art is recognized to be high (see, *e.g.*, Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986)). The numerous articles and patents made of record in this application address a highly skilled audience and further evidence the high level of skill in this art.

Knowledge of those of skill in the art

At the time of filing of the application and before, one of skill in the art knew the biochemical and structural properties of the various components of chromosomes from a wide variety of species (*e.g.*, plants, animals, bacteria and yeast). Further, there was a broad body of knowledge, set forth below and incorporated by reference into the instant specification, that was directed to the structure and function of native and artificial chromosomes from various sources (*e.g.*, animal, bacteria, yeast and plant). Also known to those of skill in the art were methods for the manipulation of DNA, recombinant DNA techniques and techniques for the transfer of DNA into cells. Numerous such procedures are referenced in the instant application, for example, as follows.

For example, sequence information for plant centromeres, telomeres and autonomously replicating sequences (ARS) was available (see, *e.g.*, Jiang *et al. Proc Natl Acad Sci USA, 93*:14210-14213 (1996); Richards, E.J. "Plant Telomeres" in *Telomeres* Eds. C. Greider, and E.H. Blackburn, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1995); Rhodes *et al. Curr. Opin. Struct. Biol. 5(3):* 311-322 (1995); Zakian *Science 270(5242):* 1601-1607 (1995); Berlani *et al. Plant Mol. Biol. 11:* 161-162 (1988); Berlani *et al. Plant Mol. Biol. 11:* 173-182 (1988); and Eckdahl *et al. Plant Mol. Biol. 12:* 507-516 (1989)). Methods for the isolation of plant centromere DNA sequences were also available (see, *e.g.*, Jiang *et al. Proc Natl Acad Sci USA, 93*:14210-14213 (1996); Kaszas *et al. EMBO J. 15:* 5246-5255 (1996); Frary *et al. Mol. Genet. 250:* 295-340 (1996)). In these methods, the centromeres are localized either by genetically analyzing the assortment of chromosome fragments and rearrangements or by cytological analysis. In the specification, *e.g.*, at page 11, line 30, through page 12, line 16, a method for cloning a centromere in a selected species (*e.g.*, plants) is described. In addition, methods for the production of repeated tandem arrays of DNA, such as telomeric DNA, are also

provided in the specification (see, *e.g.*, page 13, line 28, through page 15, line 4; and page 64, line 19, through page 68, line 27).

Procedures relating to DNA manipulation are found throughout the application as seen, *e.g.*, at page 73, line 23, through page 74, line 21, (see, *e.g.*, Sambrook *et al.* (1989) *Molecular cloning: A Laboratory Manual Cold Spring Harbor Laboratory Press,* Cold Spring Harbor, NY, Wong *et al.* (1988) *Nucl. Acids Res. 16*:11645-11661, Fatyol *et al.* (1994) *Nucl. Acids Res. 22*:3728-3736, Morgenstern *et al.* (1990) *Nucl. Acids Res. 18*:3587-3596, Tonghua *et al.* (1995) *Chin. Med. J. (Beijing, Engl. Ed.) 108*:653-659, Couto *et al.* (1994) *Infect. Immun. 62*:2375-2378, Dunckley *et al.* (1992) *FEBS Lett. 296*:128-34, French *et al.* (1995) *Anal. Biochem. 228*:354-355, Liu *et al.* (1995) *Blood 85*:1095-1103, International PCT application Nos. WO 95/20044, WO 95/00178, and WO 94/19456). These methods are not specific for the preparation of vectors and plasmids for use in a mammalian systems. Use of these methods or alteration of the methods described in the instant application to prepare vectors and plasmids for use different systems, such as, for example, a plant system, are known to those of skill in the art.

Procedures for the introduction SATACs into host plant and animal cells are referred to in many instances throughout the application. For example, the application references numerous procedures for the introduction of SATACS into cells (see, *e.g.*, at page 23, line 18, through page 24, line 11; at page 48, line 11, through page 51, line 26; and at page 54, line 1, through page 55, line 3), including the direct uptake of DNA using calcium phosphate (*e.g.*, Wigler *et al.* (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*:1373-1376), electroporation, lipid mediated transfer, *e.g.*, lipofection and liposomes, polyethylene glycol-mediated DNA uptake (*e.g.*, Strauss (1996) *Meth. Mol. Biol. 54*:307-327; U.S. Patent Nos. 4,684,611; 5,491,075; 5,482,928; and 5,424,409), microcell fusion (*e.g.*, Lambert (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*:5907-5911; U.S. Patent No. 5,396,767; Sawford *et al.* (1987) *Somatic Cell Mol. Genet. 13*:279-284; Dhar

et al. (1984) *Somatic Cell Mol. Genet. 10*:547-559; and McNeill-Killary *et al.* (1995) *Meth. Enzymol. 254*:133-152), lipid-mediated carrier systems (*e.g.*, Teifel *et al.* (1995) *Biotechniques 19*:79-80; Albrecht *et al.* (1996) *Ann. Hematol. 72*:73-79; Holmen *et al.* (1995) *In Vitro Cell Dev. Biol. Anim. 31*:347-351; Remy *et al.* (1994) *Bio Conjug. Chem. 53*:647-654; Le Bolch *et al.* (1995) *Tetrahedron Lett. 36*:6681-6684; Loeffler *et al.* (1993) *Meth. Enzymol. 217*:599-618), microprojectile bombardment (*e.g.*, Uchimiya *et al.* (1989) *J. of Biotech. 12*: 1-20; U.S. Patent Nos. 5,436,392; 5,489,520; and 5,470,708), microinjection in cells and embryos and protoplast regeneration for plants (*e.g.*, Weissbach *et al.* (1988) *Methods for Plant Molecular Biology*, Academic Press, N.Y., Section VIII, pp. 421-463; and Grierson *et al.* (1988) *Plant Molecular Biology, 2d Ed.,* Blackie, London, Ch. 7-9). Other methods described in the application for introducing SATACS into cells include nuclear microinjection and bacterial protoplast fusion with intact cells. Polycations, such as polybrene and polyornithine, also can be used.

At page 49, line 15, to page 51, line 26, the specification describes methods for introducing SATACs into particular cell types using standard techniques appropriate for each type of cells. For example, for plant cells, methods for direct gene transfer into plant cells include polyethylene glycol (PEG)-mediated DNA uptake, electroporation-mediated DNA uptake and microinjection. In addition, plants may be transformed using ultrasound treatment (see, *e.g.*, International PCT application publication No. WO 91/00358).

For example, at page 50, line 24, through page 51, line 14, the specification describes the use of electroporation for transformation of plants (see, *e.g.*, *Ag Biotechnology News 7*:3 and 17 (September/October 1990)). In this technique, plant protoplasts are electroporated in the presence of the DNA of interest that also includes a phenotypic marker. Microinjection of DNA into plant cells, including cultured cells and cells in intact plant organs and

embryoids in tissue culture and microprojectile bombardment (acceleration of small high density particles, which contain the DNA, to high velocity with a particle gun apparatus, which forces the particles to penetrate plant cell walls and membranes; see, *e.g.*, U.S. Patent Nos. 4,955,378, 4,923,814, 4,476,004, 4,906,576 and 4,441,972) have also been used. Direct transfer of DNA into cells also includes virion-mediated gene transfer methods.

Procedures for analysis of transformed cells to evaluate effectiveness of the introduction of heterologous nucleic acids are also described in the application. For example, at page 32, lines 9-11, page 38, lines 3-7, and page 70, lines 9-12, the use of *in situ* hybridization to detect the presence of specific chromosomes and/or specific DNA contained within specific chromosomes in cells is described. At page 82, lines 2-4, the use of PCR amplification and Southern blot techniques in confirming transfer of a satellite artificial chromosome into a host cell is described. At page 72, line 28, to page 73, line 22, the application references procedures for the detection and characterization of chromosomes (see, *e.g.*, Wang and Fedoroff (1972) *Nature 235*:52-54, Sumner (1972) *Exp. Cell Res. 75*:304-306, Perry and Wolff (1974) *Nature 251*:156-158, Hadlaczky *et al.*, (1986) *Exp. Cell Res. 167*:1-15, Hadlaczky *et al.* (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*:8106-8110, U.S. application Serial No. 08/375,271, Sumner (1991) *Chromosoma 100*:410-418).

At page 89, lines 1-10, the application references procedures, such as Northern analysis and assays for foreign gene products, for assessing the specificity and level of expression in cells of a gene product. In Example 6, at page 103, the application references procedures, such as absorption spectrometry and phosphocellulose paper binding assays, for assessing the level of expression of β-galactosidase and hygromycin transferase gene products encoded by a satellite artificial chromosome.

These references to numerous published information and protocols regarding plant and animal chromosomal composition and structure, DNA

manipulation, recombinant DNA expression, transfer of DNA into cells and analysis thereof for expression demonstrate the large volume of information regarding tested and reliable procedures available at the time of filing of the instant application and thus evidence the advanced state of the art at the relevant time and the availability of such procedures for manipulation of plant cells and introduction of SATACs into plant cells.

Presence of Working Examples

The specification provides numerous working examples and descriptions of the construction, isolation and transfer of SATACS from various sources, such as plant systems, into various cells, such as a plant cell. Example 1, at page 69 of the specification, describes the culture of cell lines containing various artificial chromosomes and the transfection of cells with artificial chromosomes. Example 2, at page 75 of the specification, describes in great detail the preparation and maintenance of cell lines, including EC3/75, EC3/7C5 and EC3/7C6, which contain artificial chromosomes, as well as the assays used to monitor the expression of the *neo* gene encoded by the artificial chromosomes within the cells. Example 6, at page 92 of the specification, describes in great detail, methods for the generation of cell lines containing a megachromosome and detailed structural characteristics of this satellite artificial chromosome. Example 8, at page 113 of the specification, describes in great detail the *in vivo* replication of a megachromosome. Example 10, at page 124 of the specification, describes in great detail methods for the isolation of satellite artificial chromosomes from endogenous chromosomes based upon the atypical base content and/or size of the satellite artificial chromosome. Example 12, at page 140 of the specification, describes in great detail the preparation of vectors and plasmids, such as the λCF-7 and the λCF-7-DTA vectors and the pMCT-RUC and the pLNCX-ILRUC plasmids, for the targeted integration of heterologous DNA into artificial chromosomes. Example 13, at page 165 of the specification, describes methods for the microinjection of artificial chromosomes

into eukaryotic cells, and detection of expression of the encoded heterologous DNA (βGal) in cells injected with the DNA.

Predictability

As is known to those of skill in the art (described above), the level of knowledge and skill in the preparation, isolation, manipulation and transfer of heterologous DNA and artificial chromosomes as claimed in the instant application was high as of the effective filing date. Therefore, given the extensive teachings of the specification, in combination with what was known at the time the instant application was filed, it is not unpredictable that the satellite artificial chromosomes provided herein can be modified to produce non-mammalian satellite artificial chromosomes and can be introduced into species other than mammalian cells (*e.g.*, plant cells).

The pending claims are directed to methods for producing a cell that contains heterologous nucleic acid in which heterologous nucleic acid is introduced into a cell by introducing a satellite artificial chromosome into the cell. The Office Action alleges that mammals and animals are not representative of plants in terms of chromosomes and chromatin structure. At page 6, paragraph 2 of the Office Action, the Office Action cites Ferl *et al.* (in Buchanan *et al.* "*Biochemistry & Molecular Biology of Plants*" (2000) American Society of Plant Physiologists, Rockville MD, pg. 324) and Lehninger ("*Biochemistry*" 2nd Edition (1976) Worth Publishers, New York, pg. 864) for the proposition that differences in base content of satellite DNA between plants and animals (AT-rich for animals and GC-rich for plants) result in differences in compactness of the satellite DNA. The Office Action then alleges that it is unclear how compact, densely H-bonded DNA affects satellite artificial chromosome activity and function.

The Office Action does not provide any suggestions based in the art that the composition of satellite DNA is linked to function. It is merely an observation that certain highly re-iterated sequences in plants and animals are

either GC- or AT-rich, respectively, and hence have different physical properties as measured by physical biochemical methods to determine molecular density. Applicant respectfully submits that the physical properties of a class of DNA, as measured by buoyancy density centrifugation have little to do with its functionality as an element of a chromosome. Indeed, a chromosome is comprised of multiple components, including DNA and a variety of proteins, in a specific arrangement. For a chromosome to function, all of these components must be present in a functional form. Regardless, as stated in the specification, a satellite artificial chromosome is a chromosome that is substantially all heterochromatin (see, *e.g.*, the specification at page 18, lines 23-25). Heterochromatin, by definition, is a densely packed form of chromatin (see, *e.g.*, Voet & Voet in "*Biochemistry*" (1990), Wiley & Sons, New York, pg. 1033). Hence, absent evidence to the contrary, the mere fact that GC-rich DNA is more densely packed than AT-rich DNA should not effect the structure or function of a satellite artificial chromosome, which is already formed from a high percentage of densely packed heterochromatin. Rather, the highly condensed nature of heterochromatin, irrespective of whether it is of plant or mammalian origin, reinforces the similarities between plant and mammalian satellite artificial chromosomes that would enable generation of either species by following the methods and teachings of the specification.

Further, the Examiner is reminded that the structure of a chromosome contains additional features beyond simple DNA sequences. This includes the proteins that associate with the DNA to lead to the formation of the higher order chromatin structure of the chromosome. Plant and animals both contain histone and non-histone proteins, and it has been well-established that there is a large degree of conservation between animal and plant histone proteins. In particular, it has now been established that animal and plant chromosomes contain proteins at the centromere region that are highly conserved (see, *e.g.*, Mole-Bajer *et al. Proc. Natl. Acad Sci. USA 87:* 3599-3603 (1990); and Houben *et al.*

Chromosome Res. 3: 27-31 (1995)). These are particularly important proteins that bind to centromere regions and form the functional kinetochore. A functional kinetochore is essential for chromosome movement during mitosis. Numerous examples of similarity between kinetochore proteins of animal and plant species have been reported.

It is known that the composition of the kinetochore includes the centromere DNA sequences and proteins with kinetochore function. Antibodies to human kinetochore proteins cross react with plant kinetochore proteins as labeled on mitotic chromosomes (see, *e.g.*, Mole-Bajer *et al. Proc. Natl. Acad Sci. USA 87:* 3599-3603 (1990); and Houben *et al. Chromosome Res. 3:* 27-31 (1995)). Mole-Bajar *et al.* and Houben *et al.* demonstrated cross-reactivities of human antibodies from a patient with CREST against kinetochores of mitotic chromosomes of *Haemanthus katherinae* Bak and against the centromeric regions of mitotic chromosomes of Vicia faba (bean). In both plant species, putative homologs of the kinetochore protein SKP1 (suppressor of kinetochore protein 1p of yeast) were found. Accordingly, the art suggests that the essential elements of plant and animal kinetochores are highly similar and would be expected to behave in a similar fashion.

The Office Action also alleges that telomeres, origin of DNA replication and a centromere are required for the function of a satellite artificial chromosome, citing Willard (*Science 290:* 1308-1309 (2000)). The Office Action alleges that it is unclear what telomeres, origin of DNA replication and centromere are necessary for non-animal satellite artificial chromosomes, whether additional components are required or how to isolate or construct functional satellite artificial chromosome in all cell, or non-mammalian cells (see, Office Action, page 6, paragraph 1).

Contrary to the assertion of the Office Action, the specification does provide methods for the development of a satellite artificial chromosome, which can be of animal or plant origin and comprise centromere-related sequences

(see, *e.g.*, the specification at page 12, lines 2-18; and page 61, line 4, through page 64, line 18). In addition, methods for the production of repeated tandem arrays of DNA, such as telomeric DNA, are also provided in the specification (see, *e.g.*, page 13, line 28, through page 15, line 4; and page 64, line 19, through page 68, line 27). Utilizing the teachings of the specification and the teachings known in the art at the time of filing of the instant application, it is a simple matter to develop a satellite artificial chromosome in an animal cell, modified to contain sequences specific to the telomeric, origin of DNA replication or centromeric region of a different target cell and use said satellite artificial chromosome in the target cell type.

Further, telomeric sequence, structure and function were well understood in the art at the time of filing (see, *e.g.*, Richards, E.J. "Plant Telomeres" in *Telomeres* Eds. C. Greider, and E.H. Blackburn, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1995); Rhodes *et al. Curr. Opin. Struct. Biol. 5(3):* 311-322 (1995); and Zakian *Science 270(5242):* 1601-1607 (1995)). Telomere sequences are highly conserved between animals and plants, comprising the same simple sequence CCCTAAA in humans and *Arabidopsis* (see, *e.g.*, Richards, E.J. "Plant Telomeres" in *Telomeres* Eds. C. Greider, and E.H. Blackburn, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1995); Rhodes *et al. Curr. Opin. Struct. Biol. 5(3):* 311-322 (1995); and Zakian *Science 270(5242):* 1601-1607 (1995)).

Furthermore, sequential and functional information regarding autonomously replicating sequences (ARS) in plants, which correspond to origins of DNA replication, was also known in the art at the time of filing (see, *e.g.*, Berlani *et al. Plant Mol. Biol. 11:* 161-162 (1988); Berlani *et al. Plant Mol. Biol. 11:* 173-182 (1988); and Eckdahl *et al. Plant Mol. Biol. 12:* 507-516 (1989)). In addition, the ability of an animal origin of replication to function in a plant cell was demonstrated at a functional level by Hadlaczky *et al.* (*In Vitro 16:* 647-650 (1980)) through the incorporation of label into animal DNA within a

plant cell. The specification also provides a method for generating species specific satellite artificial chromosomes by adding a centromere from other species, including plants (see, *e.g.*, page 12, lines 3-16). Further, at the time of filing, exemplary sequence information for plant centromeres was available in the art (see, *e.g.*, Jiang *et al. Proc Natl Acad Sci USA 93:* 14210-14213 (1996); Kaszas *et al. EMBO J. 15(19):* 5246-5255 (1996); Frary *et al. Mol. Gen. Genet. 250:* 295-304 (1996); and Moore *et al. Chromosoma 105:* 321-323 (1997)). The methods outlined in these references provide a means to identify and isolate plant centromere DNA sequences. For example, Jiang *et al.* utilized fluorescence *in situ* hybridization to analyze the structure and molecular organization of the centromeric sequence in *Sorghum bicolor*.

The Office Action cites Ferl *et al.* in Buchanan *et al.* "Biochemistry and Molecular Biology of Plants" (2000) American Society of Plant Physiologists, Rockville MD, pg. 324; Willard *Science 290:* 1308-1309 (2000); Shen *et al. Current Biology 10:* 31-34 (2000); and Telenius *et al. Chromosome Res. 7*: 3-7 (1999), which allegedly demonstrate that centromeres of satellite artificial chromosomes show some species-specific behavior.

Furthermore, as discussed, such knowledge is not needed to practice the methods as claimed. As described above, cells, when treated as described in the instant application generate the chromsomes from which the SATACs are generated by virtue of amplification of regions of a chromosome. As discussed above, plant chromsomes have amplifiable regions (see, *e.g.*,I U.S. Patent Nos. 6,355,860 and 6,100,092, attached hereto). Introduction of nucleic acids will result in amplification events. If the resulting cells are examined or sorted or treated and grown under selective conditions cells containing SATACs will be identifeid.

It is respectfully submitted that the references cited by the Examiner have no bearing on the predictability of the methods as instantly claimed, namely, methods for producing a cell that contains heterologous DNA by introducing a

satellite artificial chromosome into the cell. Willard provides the general state of the art with respect to components for of its artificial chromosomes and their assembly, but provides no teachings relevant to the production of SATACs. Ferl *et al.* makes a general assertion that the sequences of the centromere among species varies. Ferl *et al.* does not, however, provide any teaching that this species variation impacts on the ability to produce satellite artificial chromosomes from any species, nor their introduction into cells of different species. In fact, Shen *et al.* and Telenius *et al.*, also cited by the Examiner in this regard, show that artificial chromosomes can be transferred between a wide variety of cells (*see, e.g.*, Shen *et al.* at page 33, column 2, lines 1-5; Telenius *et al.* at page 6, clumn 2, para. 3). Therefore, the species variation of centromere sequences, if any, has no bearing on the predictability of the methods as instantly claimed.

At page 7 of the Office Action, paragraph 1, the Office Action points to Avramova (*Plant Physiology 129:* 40-49 (2002)) as an assessment of the structure and function of heterochromatin in plants in comparison to its role in animals, which allegedly notes "heterochromatin is located at the nucleolar organizer and at the chromosome knob" in plants. Avramova continues, stating that (see, page 41, right column, second paragraph):

> at least three features make plant heterochromatin different from the animal chromatin: (a) absence of proteins similar to known heterochromatin proteins (see "Note added in proof"); (b) location of potentially active genes in the knob structures and in the pericentromeric regions of plant genomes; and (c) difference chromosomal environments for colinear genes in related species.

Avramova then states that "a family of approximately 20 putative DNA methyltransferases, containing a chromobox in the putative active center, is unique for plants" (see, page 44, left column, paragraph 2). The Office Action alleges that these passages show that patterns of heterochromatin differ between plants and animals. The Office Action further alleges that these

differences coupled with differences in plant satellite DNA and plant centromeres and their animal counterparts demonstrate that "it is unpredictable that a plant satellite artificial chromosome, consisting of those components, would function as desired" in the claimed subject matter (page 7, last paragraph of the Office Action).

It is respectfully submitted that such as selective reading of Avranova, in which statements regarding the comparison of structure and function of plant versus animal heterochromatin are taken out of context, has resulted in a mischaracterization of the reference that cannot validly be relied upon to support an allegation for unpredictability of plant satellite artificial chromosomes and their function as claimed in the instant application. For example, the Office Action points to a passage that states that heterochromatin in plants is located "at the nucleolar organizer and at the chromosome knobs" (page 7, first paragraph). However, this passage begins: "In plants, **in addition to the centromeric and pericentromeric regions**, heterochromatin is located at the nucleolar organizer..." (page 40, left column). This statement, which was disregarded by the Office Action, indicates heterochromatin is also present in corresponding regions in plants and animals, specifically, the centromeric and pericentromeric regions.

Additionally, one of the differences between plant and animal heterochromatin that is cited in the Office Action, "the absence of proteins similar to known heterochromatin proteins (see "Note added in proof")" (page 41, right column) is actually a similarity (page 47, right column):

> Recent groundbreaking results in *Neurospora sp.* and *Arabidopsis* provided evidence for a connection between DNA methylation and histone H3 K9 methylation (H. Tamaru, E.U. Selker (2001) *Nature 414:* 277-283; J.P. Jackson, A.M. Lindroth, X. Cao, S.E. Jacobsen (2002) *Nature 416:* 556-560). In addition, the latter paper provided first evidence for the existence of histone H3 K9 methyulation in plants, for the activity responsible for this modification, and for its connection to plant-specific CpNpG DNA methylation. A newly

> reported HP1-like factor from *Arabidopsis* (V. Gaudin, M. Libault, D. Poteau, T. Juul, G. Zhao, D. Lefebre, O. Grandjean (2001) *Development 128:* 4847-4858) is involved in mediating the control of CpNpG DNA methylation by H3 K9 methylation (Jackson *et al.*, 2002). Collectively, **these new results transform one of the differences between animals and plants, *i.e.*, absence of reported plant heterochromatin proteins, into a similarity** (emphasis added)

These statements, which were disregarded by the Office Action, indicate that plants do possess heterochromatin proteins with similar activities to those seen in animals.

Further, the Office Action points to plants having "a family of 20 methyltransferase enzymes unique to plants" (page 7, paragraph 1 of Avramova). Avramova, however, groups these enzymes into Class B of the chromodomain proteins, which **are not components** of heterochromatin (pages 42-44).

Additionally, according to the author, there are several similarities between plant and animal heterochromatin, as stated at page 41, right column:

> In summary, (a) in most species, the DNA moiety of heterochromatin is made of methylated repetitive DNAs of difference types (including mobile elements) intermixed with low-copy and unique sequences; (b) a prerequisite for heterochromatin formation appears to be the structural orgaization of the repeats rather than the nature of the particular sequences, or their repetitive character; and (c) **based on the types and arrangement of the repetitive DNAs, heterochromatin in plants is similar to the heterochromatin in animals** (emphasis added)

The Office Action alleges that the "patterns" of heterochromatin differ between plants and animals, and correlates this difference and those described above to the unpredictability of the operability of a plant satellite artificial chromosome as claimed in the instant application. Avramova, however, indicates that the pattern of the heterochromatin is not the delineating factor in its function. Rather, the structural organization of the repeats appears to be

instrumental in the activity of the heterochromatin in plants and animals, which, according to Avramova, is similar between plants and animals.

At page 8, para. 2, of the Office Action, the Examiner cites Willard *Science 290:* 1308-1309 (2000); Brown *Trends in Biotech. 18:* 403 (2000); Perez *et al. Trends in Biotech 18*: 402-403 (2000); and Hadlaczky *Curr. Opin. Mol. Ther. 3:* 125-132 (2001)); for the proposition that "the transfer of large pieces of DNA between cells is a major problem in artificial chromosome technology."

As discussed above, Willard provides the general state of the art related to the assembly of **its** artificial chromosomes. Willard does not provide any teaching that the satellite artificial chromosomes, whose structure, generation, transfer into cells and stable expression are provided in the instant specification in great detail, would show any species-specific differences in the ability to be transferred between cells. Perez *et al.* and Hadlaczky *et al.* comment on the general inefficiency of transfer of artificial chromosomes, but then go on to say that satellite artificial chromosomes appear to overcome these problems and are especially well suited for production in different cell types. Thus, the references cited by the Examiner in fact describe how the problem of transfer of large pieces of DNA between cells can be overcome. Brown merely responds cynically to the assertions in Perez *et al.* that satellite artificial chromosomes are transferred efficiently between cells, but provides no evidence to refute the methods of Perez *et al.* As discussed above, other references cited by the Examiner (see, e.g., Shen *et al.* and Telenius *et al.*) also demonstrate efficient tranfer of large pieces of DNA between cells.

Therefore, it is respectfully submitted that a high degree of conservation between or knowledge about the essential functional elements of plant and animal chromosomes, such as the telomeres, the kinetochore and the autonomously replicating sequences (ARSs) was known at the time of filing of the instant application, to render the instantly claimed methods predictable.

Further, should a modification be required, such as alteration of the centromeric sequence, methods for modifying the mammalian satellite artificial chromosomes are disclosed in the instant application, including identifying and adding centromere DNA sequences from various organisms, including plants, and known to those of skill in the art. Thus, Applicant respectfully submits that one of skill in the art could, using the teachings of the specification and the information available in the art, identify and incorporate telomeric, centromeric and autonomously replicating sequences for use in a satellite artificial chromosome operable in a plant cell. Furthermore, methods to modify the satellite artificial chromosome described in the instant application were adequately described in the specification (see, *e.g.*, page 39, line 26, through page 40, line 21; and pages 150-157), where homologous recombination is used to insert new DNA sequences into a satellite artificial chromosome. Accordingly, methods to generate and modify a satellite artificial chromosome to contain plant telomere, centromere and autonomously replication sequences has been described.

The Office Action also alleges that the specification of the instant application does not provide guidance for the manipulation of the enabled mammalian satellite artificial chromosomes to prepare a satellite artificial chromosome operable in cellular systems other than animal cells.

To the contrary, the specification describes in great detail, methods for the addition of heterologous DNA sequences, such as DNA derived from plants, to an animal satellite artificial chromosome. Furtheremore, the methods by which the animal SATACs are generated are applicable to any eukaryotic species; these methods are not species specific.

Applicant respectfully submits that it is not a requirement *a priori* to make changes to a mammalian satellite artificial chromosome to function as claimed within a plant cell. The transfer amd function of an mammalian-derived satellite artificial chromosome in a plant system is within the scope of the methods as

described in the instant application and the knowledge of those of skill in the art. For example, methods for transferring naturally occurring chromosomes between animal and plant cells are known in the art (see, *e.g.*, Szabados *et al. Planta 151:* 141-145 (1981)). Szabados *et al.* demonstrated that isolated mitotic animal chromosomes can be transferred into plant cells by simple techniques, such as by producing a fusion product between a plant protoplast and isolated chromosomes from animal cells. Thus, the art clearly demonstrates that transfer of animal chromosomes into plant cells can be achieved. Further, numerous methods for the transfer of heterologous DNA, including artificial chromosomes, into non-mammalian cells, such as insect, avian and plant cells, are referenced throughout the instant application (see, *e.g.*, page 10, lines 25-31; page 48, line 11, through page 51, line 26; page 52, line 11, through page 55, line 3; page 70, line 14, through page 72, line 27; Example 11, beginning on page 137; Example 13 beginning on page 165; and Example 14, beginning on page 176).

The art further demonstrates that once transferred into a plant cell environment, the animal chromosome is still capable of undergoing DNA synthesis (see, *e.g.*, Hadlaczky *et al. In Vitro 16:* 647-650 (1980)). By monitoring the incorporation of ^{3}H thymidine, Hadlaczky *et al.* demonstrated that *Drosophila* nuclei were able to both synthesize DNA and divide in the mixed cytoplasm. Accordingly, at the time of filing of the instant application, the art indicates that animal chromosomes can be transferred into plant cells and that these chromosomes are operable for DNA replication and mitotic division within the plant cell. Therefore, there is precedence in the art that a satellite artificial chromosome that is derived from an animal cell can be transferred into a plant cell and be operable as claimed in the instant application. The Office Action also alleges that the Applicant teaches an animal satellite artificial chromosome in a mammalian cell but does not provide the guidance to make necessary changes

to a mammalian satellite artificial chromosome to derive a non-mammalian satellite artificial chromosome thus requiring undue experimentation.

In particular, using a satellite artificial chromosome for various applications such as construction of libraries and delivery of new genetic sequences to cells are fully enabled within the specification (see, *e.g.*, the specification, at page 61, lines 5-25). This passage describes the use of a a satellite artificial chromosom, to identify functional centromeres from other organisms. In addition, the specification also teaches methods for isolation (see, *e.g.*, page 41, line 5, through page 42, line 3; page 128, line 21, through page 135, line 21) and delivery of satellite artificial chromosomes to various cells, such as, for example, via microcell fusion in mammals (see, *e.g.*, page 70, line 15, through page 72, line 27), insect cells (see, *e.g.*, page 139, line 5, through page 140, line 10), avian cells (see, *e.g.*, page 178, line 17, through page 179, line 30). Applicant submits that it is routine procedure for one of skill in the art to adapt the methods disclosed in the instant application to effect the delivery of a satellite artificial chromosome into a cell of other eukaryotic species, such as plants. Thus, the ability to produce, modify and deliver a satellite artificial chromosome to various cell types allows for the production of cells from different species. Accordingly, the use of a satellite artificial chromosome prepared from species other than mammals and its transfer into cells from species other than mammals is described.

Conclusion

In light of the extensive teachings and examples in the specification, the high level of skill of those in this art, the knowledge of those of skill in the art, the fact that it is predictable that satellite artificial chromosomes from various sources (*e.g.*, plants) can be produced introduced into a selected cell (*e.g.*, a plant cell) and the breadth of the claims, it would not require undue experimentation for one of skill in the art to practice the methods as claimed.

Accordingly, a consideration of the factors enumerated in Ex parte Forman leads to the conclusion that undue experimentation would not be required to introduce a satellite artificial chromosome into a cell based on the disclosure in the specification.

Policy Considerations

A significant portion of the grounds for the rejection of the claims under 35 U.S.C. § 112, first paragraph, is based on the alleged unpredictability of the art of the production of satellite artificial chromosomes from any source and their introduction into any cell. The Office Action alleges that the specification does not teach the production of a "universal" satellite artificial chromsome that would be operable in any cell type. The claims are directed to the introduction of a heterologous nucleic acid into a cell, where a satellite artificial chromosome is introduced into the cell, and the specification fully enables one of skill in the art to accomplish this end result. In other words, the specification teaches one of skill in the art to "make and use" a satellite artificial chromosome for the introduction of heterologous nucleic acid into a cell. The specification provides agents, such as cell lines and vectors, and methods for the production of satellite artificial chromosomes and the transfer of the satellite artificial chromosome into a cell, and describes their use *e.g.*, in gene therapy and the production of transgenic animals and plants that possess desired traits, such as resistance to disease. In fact the application does teach a "universal" method for production of satellite artificial chromsomes and methods for transfer of such chromosomes into any cell type.

Accordingly, the issue of whether the specific instant claims are enabled by the specification should not turn on the state of the art regarding the similarities between plant and animal chromosomal composition and function, as generally discussed on pages 5-8 of the Office Action. Instead, the relevant question with regard to enablement of the subject matter of the instant claims is whether the particular steps and materials of the claimed methods are described

in the specification in such a way as to enable one skilled in the art to make and use the subject matter **as claimed**. Therefore, as discussed above, the instantly claimed methods are described in detail in the application to the satisfaction of 35 U.S.C. § 112, first paragraph.

Further, as taught in the above-captioned application, any methods known in the art pertaining to introduction of foreign genes carried in traditional, standard sources (such as genes harbored in expression vectors) into cells for any variety of purposes, *e.g.*, gene therapy, protein production and the generation of transgenic animals, can be applied in similar fashion to the introduction of satellite artificial chromosomes, such as plant satellite artificial chromosomes into cells. The application describes and demonstrates that once the artificial chromosomes are generated and isolated and/or introduced into cells, then any known procedure that has previously been carried out with any heterologous gene from any source is applicable to utilization of artificial chromosomes carrying foreign genes of interest. The application is replete with descriptions of numerous uses of satellite artificial chromosomes and minichromosomes. The descriptions of the many ways in which the artificial chromosomes can be used include references to reported procedures for introducing exogenous nucleic acids into cells.

Applicant is entitled to claims that are commensurate in scope not only with what applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the applicant has disclosed. In the above-captioned application, Applicant provides a pioneering discover and discloses to the public methods and compositions for the controlled introduction and stable extra-genomic maintenance of large heterologous DNA fragments in cells without disruption of the inherent genome and, likewise, without the otherwise uncontrollable influences that the genomic DNA can have on the expression of the heterologous DNA. The artificial chromosomes disclosed in the application can be produced and used to express

heterologous genes in cells, as is taught and specifically exemplified in the specification. It is clear that Applicant's discovery is of a pioneering nature, and, as such, is entitled to broad claim protection.

As a broad body of knowledge is available in the area of molecular biology and preparation of nucleic acid for use in the manipulation of chromosomal components, including many technical procedures covering the manipulation of DNA and recombinant DNA techniques, it would be unfair, unduly limiting and contrary to the public policy upon which the patent laws are based to require Applicant to limit these claims to a particular satellite artificial chromosome or cell type. See, e.g., In re Goffe, 542 F.2d 801, 166 USPQ 85 (CCPA 1970):

> for the Board to limit appellant to claims involving the specific materials disclosed in the examples so that a competitor seeking to avoid infringing the claims can merely follow the disclosure and

and make routine substitutions "is contrary to the purpose for which the patent system exists - to promote progress in the useful arts".

> The public purpose on which the patent law rests requires the granting of claims commensurate in scope with the invention disclosed. This requires as much the granting of broad claims on broad inventions as it does the granting of more specific claims on more specific inventions" In re Sus and Schafer, 49 CCPA 1301, 306 F.2d 494, 134 USPQ 301, at 304.

To require Applicant to further limit the claims would permit those of skill in the art to practice what is disclosed in the specification but avoid infringing claims so-limited. If Applicant is required to limit the claims to only the aforementioned satellite artificial chromosomes and cell types, then those of skill in the art could by virtue of the teachings of this application readily practice what is claimed by producing the satellite artificial chromosomes from a different source and/or introduce it into a varied cell type and practice what is disclosed in the application, but avoid infringing such limited claims. To permit that is simply not fair. The instant application exemplifies the preparation of

satellite artifical chromosomes from mammals, such as mouse chromosomes 1 and 7, and their transfer into mammalian cells, such as mouse fibroblast cells, chinese hamster ovary cells and human lymphocyte cells. Having done so, it is now routine to for others to prepare artificial chromosomes from other sources, such as plants, and prepare alternative host cells containing the satellite artificial chromosome. Those of skill in the art should not be permitted to make such minor modifications by substitution of a different source or host and avoid infringing such claims.

* * *

In view of the above amendments and remarks, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: HADLACZKY *et al.*

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MARKED UP CLAIMS (37 C.F.R. § 1.121)

Please amend claims 11, 16 and 20 as follows:

11. (Amended) The method of claim 1, wherein the SATAC is produced by a method, comprising:

introducing a DNA fragment into a cell, wherein the DNA fragment comprises a selectable marker;

growing the cell under selective conditions to produce cells that have incorporated the DNA fragment into their genomic DNA, whereby a satellite artificial chromosome is produced; and

selecting a cell that comprises [a]the satellite artificial chromosome (SATAC).

16. (Amended) The method of claim 11, wherein the cell is [a mammalian cell or]a plant cell.

20. (Amended) The method of claim 12, wherein, in the method for producing the SATAC, the cell is a [mammalian cell, a]plant cell[a fish cell, an insect cell, a reptilian cell, an amphibian cell, an arachnid cell or a rodent cell].